

# Blocking IL-1: interleukin 1 receptor antagonist *in vivo* and *in vitro*

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*Clinical and experimental evidence suggests that shock, arthritis, osteoporosis, colitis, leukemia, diabetes, wasting and atherosclerosis are mediated, in part, by interleukin 1 (IL-1). Inhibition of this cytokine has been a strategy for studying disease and for new drug development. A naturally-occurring IL-1 inhibitor (IL-1 receptor antagonist, IL-1ra) that blocks binding of IL-1 to its receptors has been cloned and produced in recombinant organisms. IL-1ra reduces the severity of sepsis, colitis, arthritis and diabetes in animals and is presently being tested in humans with arthritis, shock and myelogenous leukemia.*

Low doses ( $1-10 \text{ ng kg}^{-1}$ ) of IL-1 injected into humans cause an increase in the number of circulating neutrophils and platelets<sup>1</sup> as well as increased levels of hematopoietic stem cells. These hematological responses to IL-1 may prove useful to patients with suppressed bone marrow due to radiation or chemotherapy or during bone marrow transplantation. However, when injected intravenously into humans, IL-1 also causes fever, joint and muscle aches, increased sensitivity to pain and a state of lethargy. At higher doses ( $100 \text{ ng kg}^{-1}$  or higher), IL-1 reduces appetite, induces gastrointestinal disturbances and causes hypotension that can reach dangerous levels when more than  $300 \text{ ng kg}^{-1}$  is given<sup>2</sup>.

These experiments confirm animal and *in vitro* experiments with IL-1. It is now clear that IL-1 is in a strategic position to affect a broad range of biological systems and human diseases. These range from adjuvant activity for antibody formation to induction of a shock-like state and from bone marrow stimulation to a cytotoxic effect on the insulin-producing  $\beta$  cells in the islets of Langerhans.

Although IL-1 often contributes to the pathological process of hemodynamic shock, as well as to inflammatory and degenerative diseases, nature did not leave us without some protection. The existence of a naturally-occurring IL-1 receptor antagonist (IL-1ra) suggests that the body mounts its own response to inflammation and that synthesis of IL-1ra is a natural part of the resolution of the disease process. Recombinant IL-1ra is presently in clinical trials for treating rheumatoid arthritis, septic shock and chronic myelogenous leukemias. IL-1ra will also be studied in patients with inflammatory bowel disease, asthma and graft-versus-host disease.

IL-1ra competes with IL-1 for occupancy of the IL-1 cell surface receptors, but cannot trigger the cellular responses typical of IL-1. Unlike IL-1, intravenous injection of IL-1ra produces no symptoms or changes in biochemical parameters indicative of IL-1 activity. In fact, healthy human volunteers have received as much as  $750 \text{ mg}$  of IL-1ra ( $10 \text{ mg kg}^{-1}$ ) during a 3 h intravenous infusion without any clinical or laboratory changes<sup>3</sup>. If the IL-1ra possessed as little as one hundred thousandth the activity of bona fide IL-1, these subjects would be

significantly ill. This remarkable difference between occupancy of the IL-1 surface receptors by IL-1ra and IL-1 appears to be a first for cytokine biology. Whether there are similar polypeptide receptor antagonists for other cytokines is now being seriously asked.

## Anti-IL-1 strategies

The studies on IL-1ra take their cue from the well-known effects of IL-1. After the molecular cloning of the two IL-1s (IL-1 $\alpha$ <sup>4</sup> and IL-1 $\beta$ <sup>5</sup>), the use of recombinant IL-1 confirmed earlier studies on a variety of activities attributed to IL-1 (reviewed in Ref. 6), but also expanded our knowledge of the activities of IL-1. Today it appears that nearly every tissue or organ system is affected by IL-1 (for review see Ref. 7). Although IL-1 can be viewed as an enhancer of immune and host defense functions, the cytokine also initiates the expression of genes for molecules that trigger inflammation and tissue damage.

Because IL-1 induces biochemical and cellular changes characteristic of the type of inflammation and tissue remodeling observed in destructive joint diseases, attention has focused on drugs that block the effects of IL-1 or reduce its production. Cyclooxygenase inhibitors, the most commonly used anti-inflammatory agents, do not suppress IL-1 synthesis; in fact, the evidence suggests that the use of cyclooxygenase inhibitors such as ibuprofen, indomethacin or aspirin may enhance IL-1 synthesis. However, corticosteroids and inhibitors of the lipoxygenation of arachidonic acid metabolism reduce the transcription and/or translation of IL-1 but are non-specific since they also suppress the production of tumor necrosis factor (TNF), IL-2 and other cytokines. Any ameliorative effects of such agents in disease of humans or animals cannot therefore be attributed solely to reduced production of IL-1.

## Naturally-occurring inhibitors of IL-1 including IL-1ra

Naturally-occurring inhibitors of IL-1 have been reported in a variety of biological fluids such as serum, synovial exudates, urine and conditioned media<sup>8,9</sup>. Many of these agents are nonspecific since, in addition to affecting IL-1, they also suppress or bind to other

cytokines, particularly IL-2 or TNF. Other inhibitors of IL-1 are specific. An IL-1-specific inhibitor has been described from the monocytic leukemia line M20 (Ref. 10) and naturally-occurring antibodies to IL-1 $\alpha$  have also been described in human sera<sup>11</sup>.

William Arend and co-workers and Jean-Michel Dayer<sup>12-14</sup> independently described a 22-25 kDa IL-1 inhibitory activity. Among the unique properties of this inhibitor are its ability to block IL-1 (but not IL-2), the enhancement of thymocyte proliferation and of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis from fibroblasts. Using the partially purified inhibitor from urine, Seckinger and Dayer<sup>14</sup> showed that it could block specific binding of IL-1 to cells. This was later confirmed in studies using IL-1 inhibitor from human monocytes stimulated with IgG<sup>15</sup>. These and other early observations on the IL-1ra have recently been reviewed<sup>16</sup>.

The IL-1 inhibitory activity in monocyte conditioned medium was purified by Hannum and collaborators<sup>17</sup>, who isolated three variants with molecular masses of 22, 22 and 18 kDa; these differ in their degree of N-glycosylation. Using amino acid sequence information from these purified proteins, Eisenberg and co-workers<sup>18</sup> cloned a cDNA from a monocyte library. When expressed in *Escherichia coli*, the cDNA yielded an IL-1 inhibitory protein and it was convincingly demonstrated that this recombinant IL-1 inhibitory protein bound to IL-1 receptors but had no agonist activity. The generic term 'IL-1 inhibitor' was therefore replaced by the more specific 'IL-1 receptor antagonist' (IL-1ra). Antibodies to IL-1ra recognize the partially purified urinary IL-1 inhibitor on western blotting<sup>19</sup>, which indicates that the IL-1 inhibitory activity characterized by Seckinger and Dayer is probably due to the same protein.

The cDNA for IL-1ra has a number of interesting features. It encodes a 152 amino acid protein preceded by a classical 25 amino acid secretory leader sequence indicating that this protein takes a more straightforward pathway out of the cell than either IL-1 $\beta$  or IL-1 $\alpha$ . Thus, IL-1ra is found extracellularly in its mature form, whereas pro-IL-1 $\beta$  requires extracellular proteolytic cleavage to its mature form. Furthermore, the mRNA for IL-1ra does not contain the AUUUA sequence that has been implicated in shortening the half-life of several cytokine mRNAs. The glycosylation of IL-1ra is likely at Asn84. The cDNA sequence and biological activity of IL-1ra was confirmed by Carter *et al.*<sup>20</sup>, who subsequently isolated the same molecule from U937 cell conditioned medium.

The sequence of IL-1ra shows a striking similarity to those of IL-1 $\beta$  and IL-1 $\alpha$ , which leads to the proposal that IL-1ra is the third member of the IL-1 gene family. The concept that the IL-1ra is a member of the IL-1 gene family has been confirmed by the existence of two conserved intron-exon junctions in the genes for these three proteins<sup>21</sup>. An analysis of the genomic sequences for rodent and human IL-1ra, IL-1 $\beta$  and IL-1 $\alpha$ , which have been present for the last 75 million years, suggests that IL-1ra diverged from the common ancestor before IL-1 $\alpha$  diverged from IL-1 $\beta$ , implying an important role for the antagonist in the evolution of this cytokine family.

The close structural relatedness of the agonist and the antagonist raises the question of what determines their

different properties and whether one, or a few, of the structural differences may account for the inability of the antagonist to signal the cell of its presence. To date, attempts to detect early steps in the IL-1 signaling pathway that are also seen with IL-1ra have failed. IL-1ra neither stimulates the early activation of a protein kinase activated by IL-1 nor does IL-1ra become internalized after binding to the type I IL-1 receptor<sup>22</sup>. An explanation for the inability of IL-1ra to initiate signal transduction will come only from a comparison of the critical structures involved in the IL-1 receptor-ligand complex. Along these lines, Ju<sup>23</sup> has shown that a single change of Lys145 of IL-1ra to the corresponding Asp residue of IL-1 $\beta$  results in a mutein with 0.1-1% agonist activity in two of four IL-1 assays. The reverse change of Asp to Lys in IL-1 $\beta$  gave a mutein with some loss of agonist activity; a change of Arg to Gly<sup>24</sup> or Thr to Gly<sup>25</sup> near the amino terminal of the mature IL-1 $\beta$  has also been reported to result in a mutein with wild-type binding but loss of agonist activity. However, it is important to establish that these and other IL-1 muteins retain their ability to bind to IL-1 receptors during long-term bioassays, and are not denatured or otherwise inactivated by the conditions of *in vitro* assays.

## IL-1ra recognizes the two IL-1Rs

There are two IL-1 receptors, now indicated as IL-1R type I and type II (IL-1RtI and IL-1RtII), corresponding to the 80 kDa (Ref. 26) and 68 kDa (Ref. 27) IL-1-binding proteins on T and B cells respectively. Both are members of the immunoglobulin superfamily and are structurally related to each other. The type I receptor is found primarily on T cells, endothelial cells, keratinocytes, hepatocytes and fibroblasts, whereas the type II receptor is found on neutrophils, B cells and bone marrow cells. However, it is likely that some cells can express both types. Although the type I receptor appears to account for nearly all of the biological activity of IL-1, the two receptors may cooperate in binding and signal transduction on some cells. The initial studies revealed that IL-1 $\alpha$  and IL-1 $\beta$  recognize cell surface receptors with nearly the same affinity. Subsequent studies have shown that IL-1 $\beta$  has a greater affinity for the type II receptor, whereas IL-1 $\alpha$  has a higher affinity for the type I receptor<sup>28</sup>. Moreover, species specificity for IL-1, which was initially thought not to exist, can be shown<sup>6</sup>.

IL-1ra was initially reported not to recognize type II receptors<sup>18,20</sup> but recent studies have shown that it can recognize this receptor on a number of human cells. Human IL-1ra competes with IL-1 for binding on neutrophils<sup>29-31</sup>, myelogenous leukemia cells<sup>32</sup>, peripheral blood monocytes<sup>33</sup> and B cells<sup>29,31</sup>. However, it does not bind tightly to the dominant (p68) receptor on murine pre-B-cell lines or to freshly obtained murine bone marrow cells<sup>18,20,31,34</sup>. This failure to bind to murine cells does not appear to be a consequence of species specificity since recombinant murine IL-1ra also does not bind well to these cells<sup>31</sup>.

The affinity of IL-1ra for type-I-receptor-bearing cells is close to that of IL-1 $\beta$  and IL-1 $\alpha$ . However, for the type II receptor on Raji cells, the affinity for IL-1ra is equal to that for IL-1 $\alpha$  and is 25-30 fold lower than that for IL-1 $\beta$ . On neutrophils, the affinity for IL-1ra is also

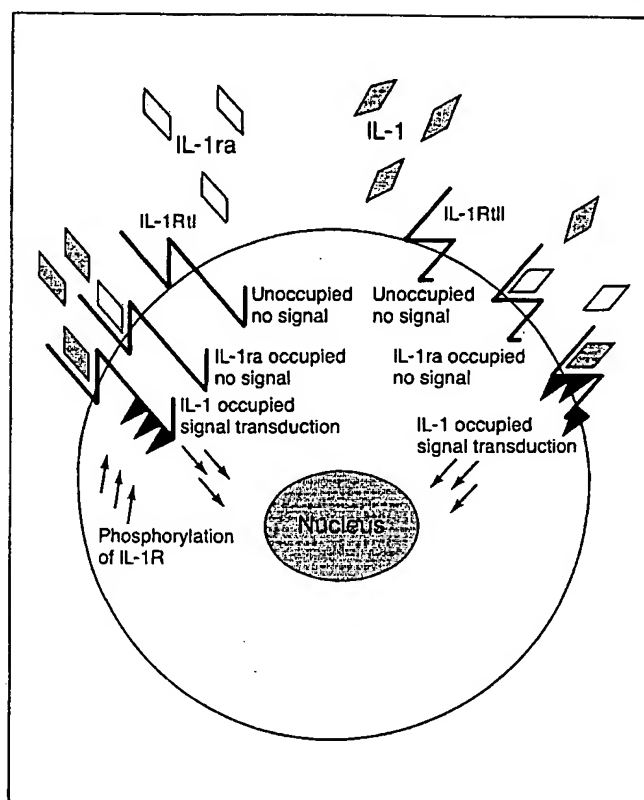


Fig. 1. Competition between IL-1 and IL-1ra for occupancy of the IL-1 receptor types I and II is shown on a cell. When IL-1 occupies either receptor, signal transduction takes place. When IL-1ra occupies either receptor, no signal transduction occurs. The large arrows symbolize proposed phosphorylations of the cytosolic segment of the IL-1R.

one-twentyfifth of that for IL-1 $\beta$  but is one two-hundredth of that for IL-1 $\alpha$ . Figure 1 shows that occupancy of either the IL-1RI or type II by IL-1ra blocks the binding of IL-1 without transducing a signal to the nucleus.

## IL-1ra blocks the biological activity of IL-1

As shown in Table 1, a large number of studies have demonstrated the ability of IL-1ra to block the activity of IL-1 both *in vitro* and *in vivo*. To date there are no examples where IL-1ra has failed to block the anticipated biological response to IL-1 added to cultured cells. Recombinant IL-1ra blocks IL-1 augmentation of thymocyte proliferation, IL-1-induced synovial cell PGE<sub>2</sub> synthesis, chondrocyte collagenase synthesis<sup>35</sup> and IL-1-induced endothelial cell adhesiveness for neutrophils<sup>20,36</sup>. IL-1ra also blocks the ability of IL-1 to activate a protein kinase in fibroblasts, an early event following the binding of IL-1 to cells<sup>22</sup>. A 10–100-fold excess of IL-1ra is required to inhibit 50% of these IL-1-induced responses *in vitro*; this is consistent with the presence of a large excess of IL-1 receptors on cells compared with the number that need to be occupied to trigger a biological response to IL-1.

IL-1ra also blocks the production of IL-1-induced IL-1, TNF and IL-6 from human monocytes. However, 50% inhibition is observed at equimolar ratios whereas at a 10-fold molar excess of IL-1ra to IL-1, complete inhibition is observed<sup>33</sup>. This observation may indicate that the 'spare receptor' effect seen with cells expressing the type I receptor is not as important as in cells expressing the type II receptor.

Recombinant IL-1ra will also block the activity of IL-1

Table 1. IL-1ra blocks IL-1-induced changes

IL-1-induced changes blocked by IL-1ra	Refs
<i>In vivo</i>	
Lethality in adrenalectomized mice	71
Circulating IL-6	71
Hypotension in rabbits and baboons	39
Fever in rabbits	72
Slow-wave sleep in rabbits	73
Cellular infiltration in cerebrospinal fluid	74
Cerebrospinal fluid IL-6 levels	71
Hypoglycemia in mice	71, 75
Hepatic acute phase proteins in mice	34
Increased corticosterone in mice	20
Peritoneal and dermal neutrophil accumulation	20,34
GABA-A receptor chloride channel in brain synaptosomes	76
Neutrophilia in mice	20,34
Release of neutrophil precursors from bone marrow in mice	34
<i>In vitro</i>	
Lymphocyte proliferation	13,15,18,20,34
Collagenase production by rabbit chondrocytes	35
PGE <sub>2</sub> synthesis in fibroblasts and synovial cells	13,35
Bone resorption in mice and rats	19
Cartilage matrix degradation	77
Adhesiveness of endothelial cells for neutrophils and eosinophils	20,36
Synthesis of IL-1, TNF, IL-6 and GM-CSF from monocytes	33
Nitric oxide production in human smooth muscle cells	78

Table 2. Reduction in severity of diseases in various models by human IL-1 receptor antagonist

Disease models	Refs
Death in rabbits from endotoxin, LPS or <i>E. coli</i>	37,38
Death in mice from LPS	40
Death in newborn rats from <i>Klebsiella pneumoniae</i>	41
Hemodynamic shock and tissue damage in rabbits and baboons after <i>E. coli</i>	38,39
Hemodynamic shock in rabbits from <i>Staphylococcus epidermidis</i>	42
Cerebral malaria in mice	79
Collagen-induced arthritis in mice	58
Streptococcal rechallenge arthritis	54
Inflammatory bowel disease in rabbits	44
Onset of spontaneous diabetes in BB rats	53
Hypoglycemia and CSF production in mice following endotoxin	75,80
Proliferation and CSF production of acute myeloblastic leukemia cells	32
Proliferation of chronic myelogenous leukemia cells	49
Neutrophil accumulation during inflammatory peritonitis in mice	34
Sciatic nerve regeneration in mice	81
Graft-versus-host disease in mice	55
Experimental enterocolitis in rats	82
Indomethacin-induced intestinal ulceration in rats	82
LPS-induced pulmonary inflammation in rats	62
Serum amyloid A protein in mice treated with high dose IL-2	83
Carrageenan-induced pleurisy	84

CSF: colony-stimulating factor.

injected into animals but, as observed *in vitro*, a large excess of IL-1ra is required to block the biological effect of the cytokine. For example, the intravenous injection of 100 ng kg<sup>-1</sup> of human IL-1 $\beta$  into rabbits induces a rapid onset of fever. To block 50% of this response, a prior injection of 10  $\mu$ g kg<sup>-1</sup> of IL-1ra (100-fold excess) is required; a complete block is observed with 100  $\mu$ g kg<sup>-1</sup> of IL-1ra (1000-fold excess). A similar dose relationship was reported for IL-1-induced hypotension in rabbits<sup>37</sup>. IL-1ra can also block IL-1-induced inflammation in mice. When injected intraperitoneally, it blocked the local accumulation of neutrophils in response to an intraperitoneal injection of IL-1. Systemic injections of 100-fold excess of IL-1ra blocked IL-1-induced circulating IL-6 levels and a 10 000-fold excess of IL-1ra blocks IL-1-induced neutrophilia<sup>34</sup>.

## The effect of IL-1ra on animal models of disease

In animal models of various diseases, IL-1 is only one of many cytokines produced during the development of different disease processes. The larger question as to what role IL-1 plays in the complex response to infection, inflammation or immune stimulation can now be studied using IL-1ra. Table 2 lists these and other models in which the effects of IL-1ra have been studied. In some of these models, IL-1ra was given just prior to the disease-inducing event but in other experiments, IL-1ra was also effective when given after the onset of disease. From Table 2, we have selected six examples to discuss in detail.

### Effect of IL-1ra on septic shock

Injection of lipopolysaccharide (LPS) or *E. coli* into rabbits produces changes typical of the septic shock syndrome, namely, hypotension, leukopenia, thrombocytopenia and infiltration of neutrophils into tissues and

tissue necrosis. Administration of IL-1ra has prevented the shock-like syndrome and death in rabbits in response to LPS<sup>37</sup> or *E. coli*<sup>38</sup>; IL-1ra was also effective if it was administered 2 h after the LPS<sup>37</sup>. Similar protective effects of IL-1ra have been observed in baboons given lethal doses of *E. coli*<sup>39</sup>, in mice given lethal doses of LPS<sup>40</sup> and in rats infected with *Klebsiella pneumoniae*<sup>41</sup>. Interestingly, the beneficial effects of IL-1ra in septic animals are not limited to sepsis due to Gram-negative organisms because IL-1ra blocks hypotension associated with staphylococcal bacteremia in rabbits<sup>42</sup>, where IL-1 has been shown to play an important role<sup>43</sup>. These results have led to a re-evaluation of the sole role of TNF in septic shock. IL-1 appears to play an essential role in the progression of the disease. The observations that TNF and IL-1 act synergistically in producing shock, the Schwartzman reaction and destruction of the  $\beta$  cells in the islets are consistent with this view (reviewed in Ref. 6).

A role for IL-1 has been proposed in the pathogenesis of inflammatory bowel disease. During immune-complex-induced colitis, the degree of inflammation, edema and necrosis in colonic tissue correlates with the tissue levels of IL-1 (Ref. 44). When rabbits were pre-treated with the IL-1ra, a marked decrease in tissue inflammatory cell infiltration, edema and necrosis was observed<sup>44</sup>.

In septic shock, in colitis and in other models of inflammation characterized by infiltration of neutrophils and other leukocytes, IL-1ra is probably blocking the ability of endogenous IL-1 to stimulate the expression of endothelial cell adhesion molecules<sup>20,36</sup> and IL-8 production. This would account for the reductions in leukopenia, accumulation of cells into inflammatory sites and

subsequent tissue and organ damage. In addition, IL-1 is a potent stimulator of nitric oxide production, which inhibits arterial wall contraction<sup>45</sup>, and blocking this IL-1-mediated event may be a major reason why IL-1ra can reduce hypotension and organ failure.

## *Effect of IL-1ra on spontaneous growth of acute and chronic myelogenous leukemia cells*

Peripheral blood mononuclear cells from healthy human donors do not express the gene for IL-1 $\beta$  or IL-1 $\alpha$  at levels detectable using the polymerase chain reaction (Margolis and Wakabayashi, unpublished). On the other hand, myeloma and Hodgkin's cells as well as B, T, granulocytic and acute myelogenous leukemic cells express IL-1 $\beta$  mRNA spontaneously and synthesize biologically active IL-1; antibodies to IL-1 $\beta$  block the spontaneous production of colony-stimulating factors and the spontaneous proliferation of these cells<sup>32,46-48</sup>. IL-1ra blocked the spontaneous proliferation and production of colony-stimulating factors in cultured peripheral blood or bone-marrow-derived acute myelogenous leukemia cells from over 25 patients<sup>32</sup>. Similar effects have been observed in the spontaneous formation of colonies in chronic granulocytic leukemia<sup>49</sup>.

## *Effects of IL-1ra on the development of diabetes*

The immune cause of insulin-dependent diabetes continues to be debated. However, seven years ago, Mandrup-Poulsen, Nerup, Bendtzen and co-workers<sup>50,51</sup> reported that IL-1 could destroy the insulin-producing  $\beta$  cells in the islets of Langerhans<sup>50,51</sup>. According to their studies, IL-1 is an effector arm of the immune-mediated destruction of the  $\beta$  cells. Natural, urine-derived IL-1ra reduced IL-1-mediated islet cytotoxicity<sup>52</sup> and chronic administration of recombinant human IL-1ra to the BB rat strain significantly delays the onset of spontaneous diabetes<sup>53</sup>. The later onset of the disease may be due to development of anti-human IL-1ra antibodies in these rats. However, these results suggest that IL-1 is playing an essential role in the pathogenesis of insulin-dependent diabetes.

## *Effect of IL-1ra on reactivating arthritis*

Arthritis in the ankle joint of a rat that has been injured by intra-articular injection of streptococcal cell walls can be reactivated by intravenous injection of subarthritogenic amounts of these same cell walls. This reactivation may be a model of the recurrence of joint inflammation observed in humans with rheumatoid arthritis. Pretreatment with IL-1ra has been shown to reduce joint swelling by 60% and there was a similar reduction in cartilage erosion<sup>54</sup>. The administration of IL-1ra 6 h after the injection of the cell walls was associated with a significant reduction in the severity of the reaction. However, limiting IL-1ra treatment to 6 h or less after the induction of reactivation arthritis enhances joint swelling<sup>54</sup>.

## *Effect of IL-1ra on graft-versus-host disease*

After a bone marrow transplant from an allogeneic donor, mice develop a graft-versus-host disease (GVHD) similar to that observed in humans. The disease is accompanied by poor hematologic and immunologic re-

constitution and increased mortality. Treatment with IL-1ra reduced mortality and improved both hematologic and immunologic reconstitution to a level two-thirds of that seen in syngeneic transplanted animals<sup>55</sup>. The implication from these studies is that GVHD is due, in large part, to the proinflammatory actions of IL-1.

## *Effect of IL-1ra on the specific immune response*

There is a considerable amount of data demonstrating the ability of exogenously added IL-1 to enhance proliferative responses of lymphocyte cultures, particularly in the presence of mitogens or antigens. In addition, several studies have demonstrated that IL-1 can be used as an adjuvant during *in vivo* immunization. In the T<sub>H</sub>2-cell line D10.G4.1, the addition of IL-1ra reduces the proliferative response to specific antigen; this implies a role for endogenous IL-1 during an immune response<sup>56</sup>.

Although the issue of IL-1ra blocking the immune response remains unresolved, available information argues against such a role. IL-1ra does not reduce the proliferative response of human peripheral lymphocytes to exogenous mitogens, antigen challenge in cells from sensitized individuals, or the mixed leukocyte reaction<sup>57</sup>. These observations are supported by short-term experiments in human volunteers receiving 3 h constant infusions of IL-1ra<sup>3</sup> and in patients with rheumatoid arthritis receiving 28 daily subcutaneous injections of the antagonist (M.A. Catalano, unpublished). In both cases, evidence of immunosuppression or of phenotypic changes in blood lymphocytes has not been observed. Daily administration of IL-1ra did not affect the formation of antibodies to ovalbumin in rats (D. Carmichael, unpublished), responses to trinitrophenol keyhole limpet hemocyanin (TNP-KLH) or changes in the cytotoxic lymphocyte response to allogeneic cells in mice (D. Faherty, unpublished). However, there is one report of IL-1ra affecting a specific immune response: anti-type-II collagen antibody levels were suppressed in mice treated with 300  $\mu$ g per day IL-1ra<sup>58</sup> and antibody levels against human IL-1ra were lower for C57BL/6 compared with DBA/LacJ mice<sup>58</sup>.

Clearly, it is necessary to examine the effect of IL-1ra on host natural defenses and immune responses in animals of different genetic makeup using long-term treatment with homologous species of IL-1ra. Nevertheless, short-term blockade of endogenous IL-1 does not impair immune function in humans. In contrast, lymphocytes from humans receiving a single dose of corticosteroids show reduced mitogen responses and changes in subset population; these changes have not been observed with short-term administration of IL-1ra.

## *Effect of IL-1 blockade on nonspecific host defense mechanisms*

IL-1 enhances nonspecific resistance to infection and inflammation (reviewed in Ref. 59). Blocking IL-1 may therefore affect the outcome of certain disease processes. For example in newborn rats infected with *Klebsiella pneumoniae*, a 5–10 mg kg<sup>-1</sup> dose of IL-1ra reduced lethality by 50% but a 20–40 mg kg<sup>-1</sup> dose significantly increased mortality compared with control rats<sup>41</sup>. In addition, receptor blockade using a monoclonal anti-IL-1R type I (35F5) antibody increased the severity of

listeriosis in mice (E. Havell and L. Moldawer, unpublished). Further experimentation will probably delineate the effect of IL-1 receptor blockade on host natural defense mechanisms.

## The balance of IL-1 to IL-1ra production

IL-1ra is synthesized in septic animals and humans with a variety of infectious or inflammatory diseases. The balance between the amount and secretion of IL-1 and its receptor antagonist may be critical in some diseases. IL-1 and IL-1ra gene expression and protein synthesis are regulated differently<sup>60-62</sup>. For example, IL-1 $\beta$  is transcribed and synthesized in cells before IL-1ra. The dysregulation in production of the agonist and antagonist in human disease has recently been studied by Rambaldi, Cozzolino and co-workers<sup>32</sup> who examined spontaneous gene expression for IL-1 $\beta$  and IL-1ra in fresh cells from patients with acute myelogenous leukemia. Cells from the 11 patients studied all spontaneously expressed the gene for IL-1 $\beta$ , whereas the leukemic cells from only one of 11 patients expressed IL-1ra after stimulation.

During experimental endotoxemia in humans<sup>63,64</sup>, in sepsis<sup>64</sup>, or in systemic juvenile rheumatoid arthritis<sup>65</sup>, large amounts of circulating IL-1ra have been found. In several studies on circulating IL-1 $\beta$  during infection in humans, levels rarely exceed 500 pg ml<sup>-1</sup> (Ref. 66). During experimental endotoxemia in humans, levels of IL-1 $\beta$  reach a maximal concentration of 150–200 pg ml<sup>-1</sup> after 3–4 h, and then fall rapidly; in the same individuals, the peak levels of IL-1ra occur after 4 h, exceed the concentration of IL-1 $\beta$  100 fold and are sustained for 12 h<sup>63</sup>. During *E. coli* sepsis in baboons, peak IL-1ra levels occur 8–10 h later<sup>64</sup>. Thus, production of a small amount of IL-1 but a large amount of the IL-1ra appears to be a natural response in some clinical situations. High levels of IL-1ra compared with IL-1 $\beta$  have also been measured in the synovial fluid of patients with rheumatoid arthritis<sup>67</sup>. *In situ* hybridizations have shown IL-1ra mRNA in the synovia from rheumatoid arthritis as well as osteoarthritis patients<sup>68</sup>.

Endogenously produced IL-1ra probably contributes to limiting the severity of disease, but may be inadequate in overwhelming infection or acute inflammation. Providing exogenous IL-1ra in some of these situations may have beneficial effects as observed in animal models.

Unlike IL-1, IL-1ra has a classical signal peptide and is secreted into the extracellular compartment, whereas in the same cell culture, only 50% of the IL-1 $\beta$  and less than 10% of IL-1 $\alpha$  is secreted. In adherent monocytes, as much as 50% of IL-1ra remains associated within the cells<sup>60</sup>. A truly intracellular form of IL-1ra without a signal peptide has been described in keratinocytes<sup>69</sup> and it is suggested that intracellular IL-1ra in these cells acts to counter the biological activity of IL-1 $\alpha$  which remains in the cytosolic compartment of keratinocytes. However, this is not the case in human monocytes stimulated with endotoxin where nearly all of their IL-1 $\alpha$  is intracellular but less than 10% of the IL-1ra remains in these cells. Nevertheless, the concept that intracellular IL-1 $\alpha$  is biologically active has received recent experimental support<sup>70</sup> and the balance of IL-1 to IL-1ra should be considered for both intracellular and extracellular compartments.

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